#11 B. Kenny 2/10/99

Case No. 808-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: **Albrecht et al**

Serial No: **08/946,138** Group Art Unit: **1634**

Filed: **7 October 1997** Examiner: **E. Campbell**

Title: **MASSIVELY PARALLEL SIGNATURE SEQUENCING BY LIGATION OF ENCODED ADAPTORS**

Declaration by Ms. Karen L. Fearon Under 37 C.F.R. 1.132

Assistant Commissioner of Patents
Washington, D. C. 20231

I, Ms. Karen L. Fearon, declare as follows:

1. After receiving a Bachelor of Science degree in Chemistry at the University of California at Davis in 1984, I entered the Masters program in Organic Chemistry at the University of Washington, where I received a Masters of Science degree in 1988, submitting a thesis on the use of peptide-based and phospholipid-based compounds as inhibitors and probes of enzyme action. From 1988 to 1989, I was a Research Scientist at Ultra Diagnostics Corporation of Seattle, Washington, where I was responsible for synthesizing a novel family of aluminum phthalocyanine fluorescent dyes and protein and drug analog conjugates of such dyes for use as immunogens and tracers in fluorescent immunoassays, respectively. From 1989 to 1992, I was an Associate Scientist in the Therapeutics Group at Applied Biosystems, Inc. of Foster City, California, where I was a member of a team that developed a highly efficient sulfurizing agent for synthesizing phosphorothioate oligonucleotides for therapeutic applications. From 1992-1994, I was a Staff Scientist at Lynx Therapeutics, Inc., where I

developed key process improvements in the manufacture of stereoregular phosphorothioate oligonucleotides and conducted in vitro assays comparing the antisense therapeutic properties of such stereoregular compounds to those of their stereorandom counterparts. From 1994 to 1997, I was Group Leader, Chemical Research and Process Development, at Lynx Therapeutics, Inc. As Group Leader, I directed and managed a group of 10 scientists who successfully developed commercially practical methods for large-scale synthesis and purification of oligonucleotide N3'→P5' phosphoramidates, a novel class of nuclease-resistant oligonucleotide analogs. From 1997 to the present, I have directed and managed the Sequencing Group of the Massively Parallel Signature Sequencing (MPSS) Project at Lynx Therapeutics, Inc. As the head of the Sequencing Group, my responsibilities include experimental process design and testing, review and interpretation of resulting data, reagent design and testing, and oversight of the Quality Control Group. Since receiving my graduate degree in Organic Chemistry, I have co-authored numerous papers in the fields of organic chemistry and chemical process development, and I am co-inventor on numerous patents, citations of which are attached as Exhibit A. I am very familiar with invention of U.S. patent application Ser. No. 08/946,138, and I am currently employed by the assignee, Lynx Therapeutics, Inc., to develop commercial products based on it. I am also familiar with the prior art references cited by the Patent Office.

2. Prior to the invention described in 08/946,138, the DNA sequencing process described in Brenner, U.S. patent '675 was practiced at Lynx Therapeutics, Inc. As decribed in the patent, the process consists of repeated cycles of ligating labeled adaptors, detecting labels, and cleaving labeled adaptors. Preferably, in Brenner '675, as well as in the present invention (see Example I of the specification), the target polynucleotides being sequenced are attached to one or more solid phase supports, such as glass beads. As currently practiced in both methods, the efficiency of each cycle of ligation-detection-cleavage averages about 75%. That is, after each such cycle, the intensity of a fluorescent signal generated by freshly ligated adaptors on a bead is, on average, only about 75% of that of the previous cycle. In the method of '675, only a single nucleotide is identified in each cycle. Thus (for example), after 16 cycles, only about 1 percent ($=.75^{16}$) of the

original signal is available. Because of this, on average, only 4-6 nucleotides of a target polynucleotide could be identified using the method of '675.

4. Many approaches were considered for improving the amount of signal available after several cycles of ligation-detection-cleavage, including a) improving the efficiency of the enzymatic steps, b) using dyes with greater fluorescent output, c) increasing the amount of target polynucleotide on beads, and d) increasing the number of nucleotides identified per cycle of ligation-detection-cleavage. In regard to the latter approach, a severe limitation was the availability of a sufficient number of fluorescent dyes for identifying pairs, triplets, or quadruplets of nucleotides at the site where an adaptor is ligated to the end of a target polynucleotide. 16 dyes would be required to identify all pairs of nucleotides; 64 dyes would be required to identify all triplets of nucleotides; and 256 dyes would be required to identify all quadruplets of nucleotides. At the time of the invention, sets of spectrally distinguishable fluorescent dyes of that size simply did not exist. An important feature of the invention was the recognition that the number of dyes required could be greatly reduced if subsets of adaptors could be distinguished using oligonucleotide tags.

5. The degree of improvement in nucleotide identification due to the invention is illustrated by the data presented in the chart attached as Exhibit B. Curve I illustrates the average values of fluorescent signal intensities measured for each of a sequence of 16 nucleotides in five populations of beads having target polynucleotides attached. Each population consisted of about 25,000 beads. The vertical bars through Curve I at each nucleotide connect the maximum and minimum average values among the five populations for that nucleotide. The signal intensities are normalized, i.e. averaged, over the values for all four nucleotides at each location in the sequence. Curve I data was produced by four cycles of ligation-detection-cleavage, as can be seen in the stepwise drop in signal level from nucleotides 1-4 to nucleotides 5-8 to nucleotides 8-12 and finally to nucleotides 13-16. Data presented in Curve I was produced in the following experiments: i) Mix 63/64 yeast cDNA, ii)-iv) Mix 42 yeast genomic DNA (different samples), and v) Mix 89-1 stimulated human THP-1. Curve II provides a comparison of results expected from the implementation of the sequencing method of Brenner '675, which requires a complete

cycle of ligation-detection-cleavage for each nucleotide identified. The significant decay of signal intensity is clearly illustrated when each cycle of ligation-detection-cleavage reduces the signal by 75%.

7. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

12/23/98 Karen L. Fearon

Date Karen L. Fearon